

6/10/2015

Aims for today:

1. Miniprep pDawn
2. Design SapI primers
3. Design Compatibility Systems
4. Pick Colonies for BioBrick, inoculate, glycerol stock
5. Colony PCR, pDawn + LacZ, Glycerol stock, miniprep
6. Redigest pDawn (with extreme controls)
7. Reattempt Ligation of pDawn+LacZ
8. Colony PCR of BioBrick Transformation
9. Run gel of colony PCR products
10. Run gel of newly digested pDawn, Overnight ligation.

Accomplishments:

1. Miniprep pDawn
2. Re-digest of pDawn (with extreme controls)
3. Re-attempt Ligation of pDawn+LacZ
4. Colony PCR of pDawn + LacZ
5. Gel extract of newly digested pDawn
6. Transformed lux box BioBrick, pDawn + lacZ, just pDawn backbone (for miniprep and
7. making our own glycerol stock) into NEB turbo again [had just cut pDawn backbone and just digested lacZ insert transformed as well as controls]
8. Plated transformed colonies and placed into incubator (will look at results tomorrow)

Aims for tomorrow:

1. Run gels of colony PCR from 6/10/15 and see if the lacZ was ligated into pDawn backbone
2. Finish designing cloning of luxbox biobrick into pACBB-eGFP (plasmid that is compatible with pDawn)
3. Pick colonies for most recent BioBrick Transformation, Inoculate, glycerol stock, miniprep
4. Colony PCR of new pDawn + LacZ transformation
5. Induce luxbox transformed E. coli with arabinose (growing up transformed E. coli to saturation, back diluting 1:100, growing up to OD600 of 0.3, then inducing with low to high arabinose concentrations)
6. If transformation results not looking good, we will need to re-digest lacZ insert and pDawn, and re-ligate pDawn into lacZ

Questions:

1. How exactly does the two-plasmid transformation work? Do we transform the NEB turbo with one and then make it competent again and transform it with the second?
2. Is there any way we can check for incomplete digestion (because the gel resolution doesn't allow us to see that, since the part we cut out is only a few bases big)?